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Histopathological study for evaluation of trematode larval infection in the carpet shell clam, *Tapes decussatus*, from three Egyptian clam fisheries

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Digenea

Abstract A histopathological evaluation of the carpet shell clam, *Tapes decussatus* collected from three Egyptian clam fisheries [El-Max (Alexandria), Timsah Lake (Ismailia) and Ezbet El-Borg (Damietta)] was conducted over a twelve months period (December 2010–November 2011). Total of 2160 clams (shell length = 17–51.5 mm) were examined for infection with trematode larvae. Smear preparations and histopathological examination (n = 30 and 30/site/month, respectively) showed maximum prevalence (% clams infected) of 81% and 65% in Timsah Lake in May and April, respectively. Maximum infection intensity (% area of transverse tissue section occupied by parasites, n = 60 slides examined per site) was observed in the May samples of Timsah (78%), followed by El-Max (45%). Infection prevalence and intensity differences among sites were insignificant but differed significantly with season ($P < 0.05$) with maximum value in spring. Infection prevalence and intensity were significantly higher in females than in males (31.4% and 16.9%, respectively) and were synchronized with the spawning season. Digenean larvae caused a wide range of damage to clam gonads. Castration occurred in 15.1% of clams due to heavy infection and led to reproductive failure during spawning trials. Infection intensity increased with clam size (more in clams $\geqslant$31.9 mm SL with ripe gonads).

Introduction

There is rising demand for economically important edible mollusk species (e.g. oysters, clams, mussels and cockles) for local Egyptian market and for export (FAO, 2004). Most Egyptian bivalve fisheries are contaminated with pollutants, especially heavy metals and sewage driven pathogens which render bivalves less suitable for human consumption (Abdel-Moati, 1991a,b; El Deeb and Aboul-Naga, 2002; Zyadah et al.,

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2004). Therefore, there is recent increased interest in bivalve aquaculture to provide alternative pathogen- and pollutants-free source of bivalves. Concomitant with starting bivalve aquaculture, attention should be paid to monitoring the health status in natural fisheries for: (1) site selection for potential grow-out of hatchery produced bivalves; (2) early detection of health problems that might affect bivalve production such as reproductive failure and mortality; (3) assessment of pathogens that exert great hazards to human health since there is a safety concern regarding the local consumption and export of bivalves from Egyptian fisheries.

The carpet shell clam, *Tapes decussatus* is one of the most popular and commercially important mollusks in many areas of the world as well as in Egypt (Prado-Alvarez et al., 2009). Therefore, it was selected as potential candidate for bivalve aquaculture in Egypt. However, evaluation of clam health in natural fisheries is needed to assess the current status of clam fisheries and for site selection for potential clam aquaculture. Clam fishermen in Egypt claim that clam populations have declined across the Egyptian fisheries in the past decade (personal communication). This is mostly attributed to overexploitation, pollution, parasites and lack of proper fisheries management (Hanafy et al., 1997; El-Gamal, 2010; El-Sikaily et al., 2004). Mass mortality of bivalves has been recorded to be associated with physical, chemical and biological factors (Sinderman, 1990) such as high temperature (Perdue et al., 1981; Cheney et al., 2000), parasites (Lee et al., 2001), water quality and pollution (Cheng, 1993; Weiss et al., 2007). However, there is lack of information on incidences of bivalve mass mortality in Egypt. Only claims about the continued decline of production by fishermen and clam farmers who depend on collection of seeds from natural fisheries (personal communication).

Infection of bivalves with parasites may represent an important reason for decreased production either by mass mortality (e.g. mortality caused by *Perkinsus* sp.) or by slow growth, decreased fecundity or complete reproductive failure that can be caused by parasitism with digenean larvae sporocysts and cercaria of trematodes (Taskinen et al., 1994; Hanafy et al., 1997; Khamdan, 1998; Taskinen, 1998; Nago and Choi, 2004; Lee et al., 2001; Cremonte et al., 2005). Bivalves represent first intermediate host for trematode larvae especially Digenea (Lauckner, 1983; Cremonte et al., 2001). Sporocysts and redia develop in the bivalve tissue and produce enormous number of infective stage (the cercaria) which causes damage to tissues (Ramadan et al., 2010).

The present study is a histopathological evaluation of the commercially important carpet shell clam, *T. decussatus* in its main fisheries in Egypt (Alexandria, Ismailia and Damietta) with regard to infection with digenean larvae of trematodes. The study includes the monthly prevalence and infection intensity examination and their effect on clam gonads to explain the

Figure 1 Sample collection sites (red arrows) from three Egyptian cities: (A) Alexandria (El-Max); (B) Ismailia (Timsah Lake); (C) Damietta (Ezbet El-Borg).

Table 1 Monthly infection prevalence with trematodes larvae of the carpet shell clams *Tapes deccusstatus* Collected from El-Max, Alexandria.

Month-year	Number (*N*) and SL (mm) of examined clams (*N* = 60)					Trematode Prevalence (%)	SL (mm) of Infected clams		% of infected from each sex[b]		
	N	Average	St. dev.	Min	Max	% Infected	Min	Max	Males	Females	Unknown
December-10	60	32.9	3.0	25.9	39.1	25	32.97	35.03	11.1	50	20
Janusry-11	60	27.2	1.7	25.2	33.5	35	25.83	29.17	33.3	37.5	33.3
February-11	60	31.5	6.0	18.4	42.8	25	28.41	41.65	11.1	50.0	0
March-11	60	31.9	4.8	23.1	41.8	15	25.9	26.9	12.5	16.7	0
April-11	60	29.7	1.4	26.3	33.1	25	30.13	31.24	0	55.6	0
May-11	60	36.6	6.1	22.8	51.5	25	22.77	47.2	10.0	40.0	0
June-11	60	37.2	4.8	29.8	48.6	50	31.43	43.29	50	50.0	0
Jul-11	60	29.2	7.3	20.0	45.3	5	29.34	29.44	0.0	14.3	0
August-11	60	28.1	4.0	21.4	36.8	15	26.78	28.65	10.0	20.0	0
September-11	60	31.5	4.1	25.6	42.0	25	26.85	33.92	28.5	23.1	0
October-11	60	35.5	4.1	28.4	42.3	25	28.41	41.09	12.5	33.3	0
November-11	60	27.6	4.1	18.6	36.6	20	24.83	28.71	20.0	28.5	0
	720	31.6	4.3	18.4	51.5	24.2	22.8	47.2	16.6	34.9	4.4
[a]	Total	Average	Average	Min	Max	Average	Min	Max	Average	Average	Average

[a] The lower row describes the value above it as calculated from the 12 months combined.
[b] The percentage represents the percentage infected compared to number of males or females examined not to the total number of specimens.

possible impacts of these pathogens on the clam population in the study areas as potential aquaculture grow-out sites.

Materials and methods

Sampling locations

Three study stations were selected in three locations from three Egyptian cities (in the map below) that represent the main natural fisheries of Tapes clams namely; (1) El-Max (next to El-Max Lighthouse or El-Fanar) in Alexandria; (2) Timsah (in Timsah Lake off El-Ta'awen Beach) in Ismailia and (3) Ezbet El-Borg (in a clam farm) in Damietta (Fig. 1A–C, respectively).

Clam sampling, smear and histological preparation

From each station, thirty clams (shell length $\geqslant$ 17 mm) that represent market size clams were examined by smear preparation for sex determination and infection with parasites (digenean larvae). Another thirty clams/per site were examined for histopathological changes in different gonads and other tissues and for taking photos. Clams for both smear preparation and histopathological examination were initially measured for shell

Table 2 Monthly infection prevalence with trematodes larvae of the carpet shell clams *Tapes deccusstatus* Collected from Timsah Lake, Ismailia.

Month-year	Number (*N*) and SL (mm) of examined clams (*N* = 60)					Trematode Prevalence (%)	SL (mm) of Infected clams		% of infected from each sex[b]		
	N	Average	St. dev.	Min	Max	% Infected	Min	Max	Males	Females	Unknown
December-10	60	25.9	3.3	21.1	32.5	0	–	–	0	0	0
January-11	60	33.0	5.9	26.0	51.0	20	33.9	38.4	33.3	0.0	0
February-11	60	28.5	5.3	20.3	43.4	30	21.9	43.4	77.8	90.0	0
March-11	60	31.1	4.2	25.6	37.6	30	25.6	36.3	20	40	0
April-11	60	31.3	3.7	26.1	43.7	65	28.1	37.1	50	87.5	0
May-11	60	29.9	5.2	18.2	40.0	81	18.2	40.0	33.3	42.8	0
June-11	60	26.2	4.5	19.8	49.2	40	20.2	29.5	30.7	57.1	0
July-11	60	25.2	4.4	17.0	41.5	30	20.6	31.0	20.0	40.0	0
August-11	60	22.8	2.7	18.4	29.9	25	18.4	27.0	12.5	33.3	0
September-11	60	30.3	3.5	22.8	39.6	0	–	–	0.0	0.0	0
October-11	60	25.7	1.9	23.1	31.0	0	–	–	0.0	0.0	0
November-11	60	28.6	4.3	21.8	38.6	10	27.7	28.0	0.0	20.0	0
	720	28.2	4.1	17.0	51.0	27.6	18.2	43.4	23.1	34.2	0.0
[a]	Total	Average	Average	Min	Max	Average	Min	Max	Average	Average	Average

[a] The lower row describes the value above it as calculated from the 12 months combined.
[b] The percentage represents the percentage infected compared to number of males or females examined not to the total number of specimens.

Table 3 Monthly infection prevalence with trematodes larvae of the carpet shell clams *Tapes deccusstatus* Collected from Ezbet El-Borg, Damietta.

Month-year	Number (*N*) and SL (mm) of examined clams ($N = 60$)					Trematode Prevalence (%)	SL (mm) of Infected clams		% of infected from each sex[b]		
	N	Average	St. dev.	Min	Max	% Infected	Min	Max	Males	Females	Unknown
December-10	60	32.4	0.9	30.8	34.1	0	–	–	0	0	0
January-11	60	38.1	2.7	34.0	43.2	15	35.4	39.1	16.7	8.3	0
February-11	60	35.4	1.5	31.2	38.5	40	31.2	37.0	36.3	44.4	0
March-11	60	31.6	1.7	29.1	34.6	20	30.2	34.6	0.0	30.7	100
April-11	60	35.5	2.2	28.5	38.7	30	35.8	38.1	21.4	50.0	0
May-11	60	36.9	2.7	30.9	43.5	25	34.8	41.1	28.5	16.7	0
June-11	60	39.7	2.7	26.0	45.1	55	36.2	45.1	40	70.0	0
July-11	60	33.4	5.1	20.8	42.5	0	–	–	0.0	0.0	0
August-11	60	38.0	2.5	32.3	42.7	20	32.3	40.6	9.1	37.5	0
September-11	60	38.5	3.7	32.6	49.2	0	–	–	0.0	0.0	0
October-11	60	31.6	1.4	29.0	34.0	10	30.6	30.9	0.0	25.0	0
November-11	60	36.2	3.8	29.7	43.7	0	–	–	0.0	0.0	0
	720	35.6	2.6	20.8	49.2	17.9	30.2	45.1	12.7	23.6	8.3
[a]	Total	Average	Average	Min	Max	Average	Min	Max	Average	Average	Average

[a] The lower row describes the value above it as calculated from the 12 months combined.
[b] The percentage represents the percentage infected compared to number of males or females examined not to the total number of specimens.

length using digital Vernier caliper. Clams were preserved in Davidson's solution fixative for further histology processing.

Histology

The histological technique was as follows: whole clam soft tissue was removed from the shell, transverse section was cut in the middle of the body to allow fixative to penetrate tissues and tissue was preserved in Davidson's fixative solution for 24 h, dehydrated with graded ethanol, cleared with xylene, embedded in paraffin wax and sliced to 5 μm transverse sections at different organs using KD-2258 rotary microtome and stained with Harris' Hematoxylin–eosin and examined under light microscope. Photos were taken using microscope-equipped digital camera.

Prevalence and infection intensity of trematode larvae

The infection prevalence (% proportion of infected individuals to the total number examined) was calculated by dividing number of clams with parasite infection or pathology prevalence by the number of clam population examined and multiplying the product by 100.

The infection intensities (% area of transverse tissue section occupied by parasites, $n = 60+$ slides, at least 2 slides were examined for each of 30 samples examined per site) was assigned semi quantitative scales based on the extensiveness of the affected area according to Kim and Powell (2007) by giving rank numbers of 0–4 according to the area occupied by parasites as follows: 0 = no infection; 1 = light infection intensity

Table 4 Monthly variations of clam infection intensity collected from three different Egyptian fisheries and infected with varying trematode larval intensities per tissue area. Values represent the% number of clams that belong to each category (heavily, moderately or lightly parasitized) to total number of clam population examined from El-Max-Alexandria, Timsah Lake-Ismailia and Ezbet El-Borg-Damietta.

Month	El-Max				Timsah				Ezbet El-Borg			
	Prevalence%	Heavy	Medium	Light	Prevalence%	Heavy	Medium	Light	Prevalence%	Heavy	Medium	Light
December	25	5	5	15	0	0	0	0	0	0	0	0
January	35	30	5	0	20	15	0	5	15	5	5	5
February	25	10	10	5	80	30	35	15	40	25	10	5
March	15	5	10	0	30	5	25	0	20	5	10	5
April	25	20	5	0	65	0	55	10	30	5	10	15
May	25	5	10	10	40	25	15	10	25	0	25	0
June	50	10	30	10	50	10	20	10	55	0	35	20
July	5	0	0	5	30	0	15	15	0	0	0	0
August	15	5	5	5	25	10	10	5	20	20	0	0
September	25	10	10	5	0	0	0	0	0	0	0	0
October	25	25	0	0	0	0	0	0	10	5	0	5
November	20	5	10	5	10	0	5	5	0	0	0	0
Total average	24	11	8	5	29	8	15	6	18	5	8	5
St. dev.	11.0	9.3	7.8	4.8	26.0	10.5	16.9	5.7	17.6	8.4	11.4	6.6

for < 25%; 2 = moderate infection intensity for 25–75% and 3 = heavy for > 75% area occupied by the parasite. When overall weighed infection intensity for certain population (e.g. clams of certain site or shell length or sex) needed to be compared, the infection intensity numbers were multiplied by the number of clams in each infection level.

Results and discussion

A total of 2160 clams (shell length ⩾ 17 mm) were examined from the three sites that were investigated in the present study. Clams average shell lengths used in the present study were 31.6 ± 4.3 mm (min 18.4–max 51.5 mm) in El-Max, Alexandria (Table 1); 28.2 ± 4.1 mm (min 17–max 51 mm) in Timsah Lake, Ismailia (Table 2); and 35.6 ± 2.6 mm (min 20.8–max 49.2 mm) in Ezbet El-Borg, Damietta (Table 3).

Infection of T. decussatus with trematode larvae

Smear preparations of *T. decussatus* collected from the three stations during December 2010–November 2011 showed infection of clams by different types of the parasitic trematode larval stages. Clams were infected with digenean larvae, sporocysts, redia of cercaria and cercaria that was the most prominent and could be isolated from the smear preparation (Fig. 2).

Description of digenean larvae stages inside the clam gonads (Fig. 2)

Sporocyst of cercaria from *T. decussatus* contained germinal ball and immature redia. Redia (Fig. 2A) were sausage-shaped, brownish thick wall. Each redia was filled with germinal balls and developing cercariae. Redia contained birth pore, developing cercaria, germinal ball, gut, and pharynx. Cercaria contained; excretory canal, genital primordium, intestinal caeca, tail, oral sucker, ventral sucker. The tails are unforked slender shape with setae (24–27 pairs of setae arranged laterally along the tail) (Fig. 2B and C).

Although larvae were not identified in the present study, they resemble to great extent *Cercaria lata* that was isolated from *T. decussatus* and described by Ben Abdallah et al. (2009) and Hamza (2010), except for the color, it was brown in the herein redia, while it was colorless as described by Hamza (2010).

Earlier studies showed infection of *T. decussatus* with trematode larva from different families. For example, *Cercaria lata* (Digenea, Faustulidae) was first discovered and named by Lespès (1857) in *Tapes decussata* (L) collected from the Arcachon basin. Similarly, Ben Abdallah et al. (2009) isolated *C. lata* from *T. decussatus* in Tunisia. Hanafy et al. (1997) isolated cercaria of the family Lepocreadiidae from the gills of *Venerupsis decussatus* in Timsah Lake, Egypt. Finally, Ramon et al. (1999) isolated *Bacigger bacciger* (Fellodistomidae) that were later identified as *C. lata* using molecular techniques from *Donax trunculus* collected from the western Mediterranean.

Prevalence% of larval trematodes

Infection prevalence was examined in two ways in the present study using both smear preparation and histology using 30 clams for each to take the average values. Data showed less sensitivity of smear preparation at low infection intensity. Although histology was not quantitative in measuring infection intensity, it was convenient for prevalence detection. It still gave good indication on the infection intensity especially with the ability to determine clam sex and reproductive stage when infection intensity was not heavy enough to cause complete degenerations of gonad and castration. Therefore, we used histology, together with smear preparation, to determine the prevalence of trematode larval infection in the clams.

Prevalence results (the percentage of infected clams among the examined population) over twelve month period are presented in Fig. 3. Varying degrees of prevalence were observed in the three examined stations which ranged from 5% to 50% in El-Max; 0% to 81% in Timsah Lake; 0% to 55% in Ezbet El-Borg (average prevalence over 12 months were 24.2%, 27.6% and 17.9%, for the three sites, respectively).

Prevalence% by site and season

Trematode larvae started to appear in December in El-Max and in January in the other two sites, starting with low concentrations (15–35% prevalence in Timsah and Ezbet El-Borg, respectively). Maximum prevalence of 81% was observed in May in Timsah Lake, followed by 65% prevalence in April in the same site. Infection decreased gradually and disappeared in September in Timsah Lake and Ezbet El-Borg but not in El-

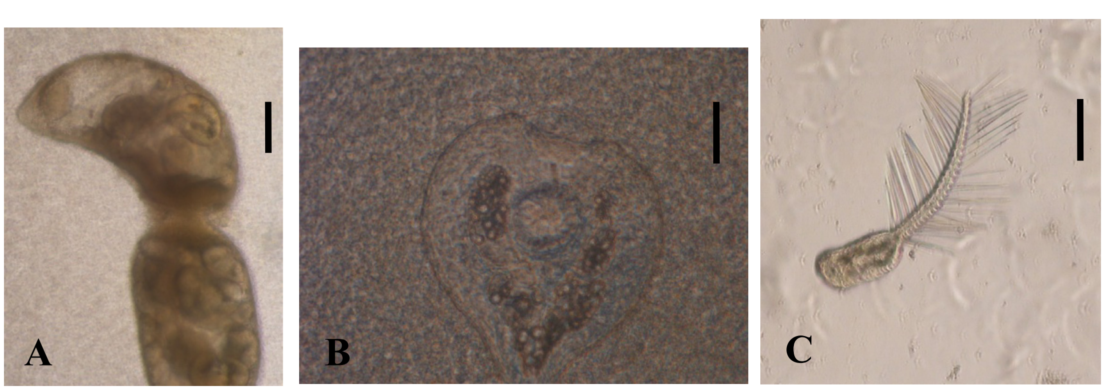

Figure 2 Photomicrographs of digenean larval; (A) redia (scale bar = 200 μm) and (B and C) cercaria (scale bar = 50 and 200 μm, respectively) and that were isolated from smear preparation of the gonads of the carpet shell clams, *Tapes decussatus*.

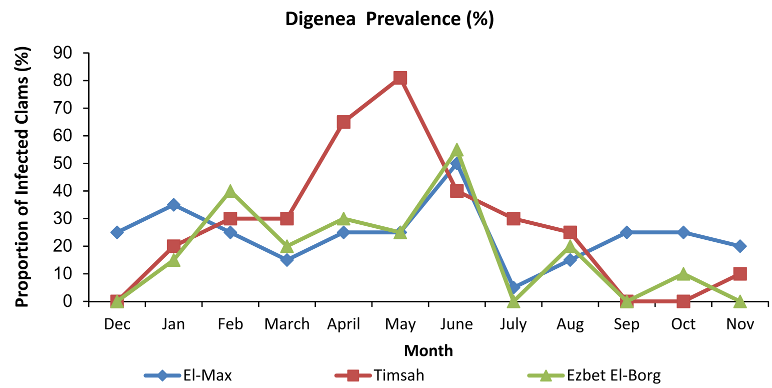

Figure 3 Monthly variations in the% prevalence of trematode larva (sporocysts, redia and cercaria) in the carpet shell clam, *Tapes decussatus* collected from three sites along the Egyptian coasts.

Max, that showed trematode larval prevalence throughout the year 5–50% (Fig. 3; Table 3).

Two-ways ANOVA showed insignificant spatial variations among sites although Ezbet El-Borg, Damietta showed lower overall prevalence average of 17.9% as compared to 24.2% for El-Max and 27.6% for Timsah Lake.

In contrast, there was temporal variation affected by the high prevalence in Timsah Lake clams especially in April and May (highest prevalence in spring). The high prevalence in the spring in Timsah Lake may be attributed to temperature change, fluctuating salinity, pollution, clam density or gametogenic cycle. However, average temperature did not show big differences among sites in the spring (24.5, 20.11 and 23.54 in Timsah Lake, El-Max and Ezbet El-Borg, respectively) to explain the highest prevalence in Timsah Lake.

In similar study, Nago and Choi (2004) in Korea showed no relation to season and recorded much lower prevalence of cercaria of 0–12% in different seasons. Additionally, Ramon et al. (1999) found no relation between infection and temperature, salinity or season.

Water salinity in Timsah Lake ranged over the study period from 15.54‰ to 40.42‰ with minimum values observed in the spring. In contrast, salinity ranged from 35.3‰ to 38.81‰ in El-Max and from 39.0‰ to 40.81‰ in Ezbet El-Borg. Timsah Lake had more salinity fluctuation due to freshwater received from the River Nile and agricultural activities in Ismailia City (Emara and Belal, 2004) into an enclosed area. In contrast, Salinity of El-Max and Ezbet El-Borg were within the normal values for their geographical range. Park and Choi (2001) observed high prevalence and infection intensity of *Perkinsus* sp. in manila clams from the west coast of Korea (where salinity widely fluctuated) as compared to the east coast in which the salinity remained constant all over the year.

Another possible reason is the elevated contamination of Timsah Lake site due to its close proximity to El-Mahsamah Drain and exposure to variety of contaminants from agricultural drainage, domestic wastes and shipping activities that dump large amounts of sewage and nutrients to the lake. This may enhance the proliferation of trematodes or their final host, especially in spring and summer seasons with increasing tourism activity. However, with this respect, El-Max is also highly contaminated site that receives huge amounts of pollutants from the metropolitan area of Alexandria that covers 40% of the nations industry via Mariut Lake (Hamza and Gallup, 1982; Hamza, 1983). Furthermore, Alexandria is the main summer resort in Egypt which increases the load of untreated sewage and waste water discharge (Abdel-Moati, 1991a,b). This renders Ezbet El-Borg the cleanest site in the present study and may explain the site's lowest infection prevalence and intensity or probably the lack of final host. Another pos-

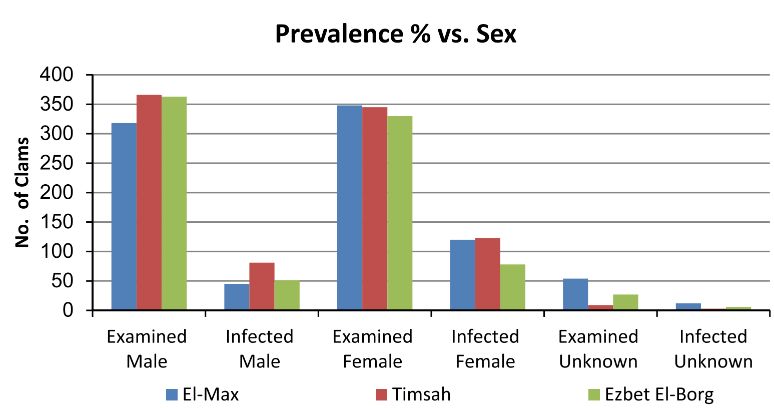

Figure 4 Total infection by trematode larva (sporocysts, redia and cercaria) of different sexes of the carpet shell clam, *Tapes decussatus* collected from three sites along the Egyptian fisheries. Values represent numbers from the three sites combined. Females showed significantly higher values of infection ($P = 0.025$).

sibility is that this site being a managed clam farm with lower clam density might be the reason of its lowest spread of infection as compared to natural beds.

The highest prevalence matched with the clam spawning season that started in April and ended in June according to El-Wazzan et al. (2012) in a study conducted simultaneously with the current study. The heavy infection associated with the spawning season have been explained by higher contents of glycogen in the ovaries of the females which provides nutrition to the trematode larvae that grow faster at the expense of the host until it causes complete castration or stunted growth of the host which exerts negative effect on production (Cheng and Synder, 1962).

Prevalence% by sex

A total of 2160 clams were examined for trematode larvae infection. Out of this number, 1047 clams were males of which 177 clams were infected (16.9% prevalence); 1023 females of which 321 clams were infected (31.4% prevalence) and 90 clams were of unknown sex of which 21 clams were infected (23.3% prevalence). There was significant difference ($P < 0.05$) in infection prevalence of males, females and unknown sex clams, females showing the highest prevalence. The infection of different sexes in different stations showed the same trend when individual sites were examined but with different values among sites, Ezbet El-Borg showed the least infection of 24% in females. Infection prevalence (%) over the 12 month period was 14%, 22%, 14% in males and 34%, 36%, 24% in females and 12%, 3%, 6% in the unknown sex for El-Max, Timsah and Ezbet El-Borg, respectively (Fig. 4).

Ramon et al. (1999) showed lower prevalence of trematode larvae in wedge clam *Donax trunculus* than those observed in the present study. In the study of Ramon et al. (1999), prevalence of 6.56%, 8.04% and 13.47% were observed in males, females and unknown sex of the *D. trunculus*, respectively with insignificant differences between males and females. In contrast, unknown sex showed significantly higher values than those with known sex (Ramon et al., 1999).

Comparing these data with those presented in the present study shows that the three study sites are considered highly contaminated with parasites as compared to other areas in the world such as Spain as in the study of Ramon et al. (1999). Therefore, the studied sites might not be suitable as grow-out sites for hatchery reared clams. However, the sites could be used as sources of broodstock taking into consideration collecting clams before the outbreak of parasite infection in the spring to avoid negative effect on reproductive potential in the hatchery.

Histopathology

Histopathological examination of samples from different locations were conducted to (1) determine specimen sex and developmental stage; (2) examine the presence of parasites especially the digenean larvae that affect the clam production either by infecting gonads and decreasing fecundity or castration and complete reproductive failure or eventual death; (3) confirmation of the prevalence data obtained from smear preparation

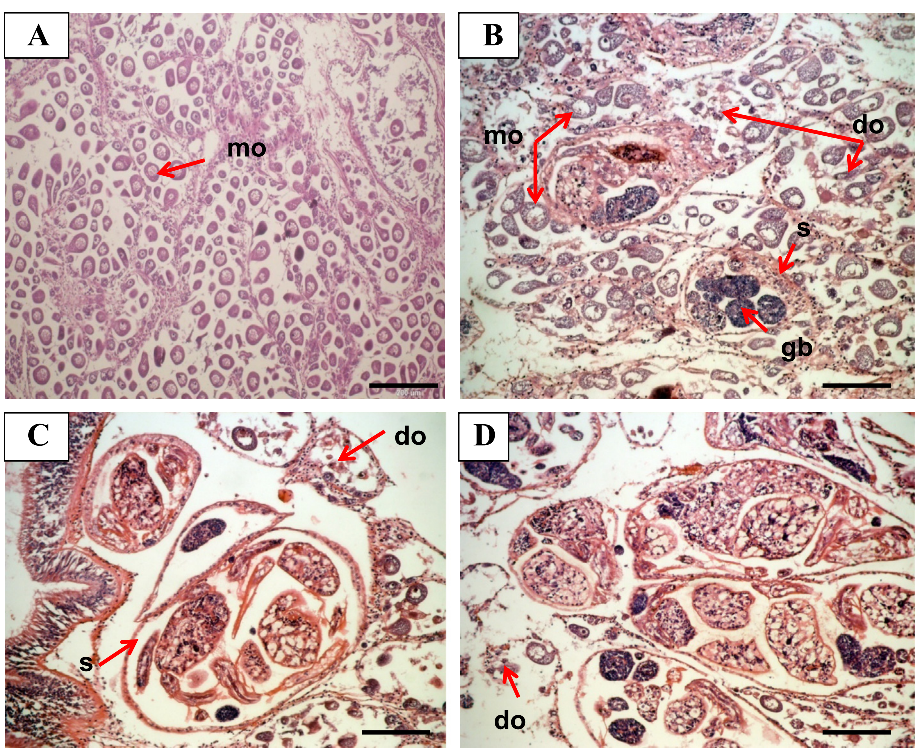

Figure 5 Photomicrographs of transverse section through the ovary of *Tapes decussatus* (A), normal uninfected ovary (scale bar = 200 μm), (B), (C) and (D), show ovaries with light, moderate and heavy infections by cercaria larvae (scale bars = 100, 50, 50 μm, respectively). *Abbreviations:* mo, mature oocyte; do, degenerating oocyte; gb, germinal ball; s, sporocyst.

and evaluates the infection intensity and the possible damage to clam tissue.

Different studies used different histology-based numerical scales to measure the infection intensity of different parasites (Ellis et al., 1998; Nago et al., 2003) to assess the effect of variable levels of infection on the host tissues.

Several ranking systems have been used for ranking infection prevalence and intensity in marine bivalves. For example, Ray (1954) system have been used for estimating the percentage of tissue occupied by the parasite according to 4 categories for the infection of parasites in bivalves using numerical ranking (0 = no infection, 1 = light infection, 3 = moderate infection and 5 = heavy infection). Kim et al. (2006) used similar ranking as semi-quantitative scale for trematode sporocyst infection in bivalves according to score description as follows: "0 = uninfected; 1 = present in the gonads only (some gametic tissue still present); 2 = completely filling the gonads (no gametic tissue present); may be present in digestive gland or gills in very limited amount; 3 = completely filling the gonads; extensive invasion of the digestive gland and/or the gills; 4 = completely filling the gonad; substantially filling the digestive gland or gill; individuals appear to be a sac of sporocyst".

In the present study, infection intensity was classified into three levels (Table 4, Fig. 8) according to the area of tissue of clam transverse sections occupied by parasites into; (1) light (<25%), medium (26–75%), and heavy (>75%). The three levels of parasites infection were suitable enough to fulfill the main purpose of the study which is assessing the possible effect of parasites on clam gonads reproductive potential for future broodstock collection for aquaculture. Therefore, the infection intensity in the present study was mainly ranked for gonads. At least 60 slides were examined per location per months.

Infection intensity of larval trematodes

The selection of histology as indicator of disease and contamination was based on previous studies that showed strong relation between pollution and the histology of gills, mantles, digestive glands and gonads (Sunila, 1986; Berthou et al., 1987; Bright and Ellis, 1989; Domouhtsidou and Dimitriadis, 2000; Sokolova, 2004; Sokolova et al., 2004; Sokolova et al., 2005).

Furthermore, histopathological biomarkers have been used to monitor parasites that negatively affect bivalve fisheries and aquaculture industry in many countries such as Egypt (Hanafy et al., 1997; Ramadan and Ahmad, 2010; El-Gamal, 2010), Korea (Park and Choi, 2001), Canada (McGladdery and Stephenson, 1996), and Argentina (Cremonte et al., 2005).

Maximum infection intensity (% area of transverse tissue section occupied by parasites, n = 60 slides examined per site) was observed in the May samples of Timsah (78%), followed by El-Max (45%). Histopathological examination showed that in the clam infected with redia or cercaria, infection was limited to gonads (Figs. 5 and 6), and in few cases to the digestive gland (Fig. 7, Fig. 8). Similar study on *D. trunculus* showed definitive localization of trematode larvae in gonads (El-Gamal, 2010).

Percentage of heavily infected clams was highest in El-Max as compared to Timsah lake and Ezbet El-Borg (Fig. 8). Heavy infection was more observed in female clams as compared to males and clams of unknown sex (Tables 1–3). For example, in El-Max, 34.9% of examined females were infected as compared to 16.6% and 4.4% of the examined males and those with unknown sex (Table 1). Similarly, the values in Timsah Lake were 34% vs. 23.1% and 0%, respectively (Table 2). In Ezbet El-Borg, the values were 23.5% vs. 12% and 8.3%, respectively (Tables 3). Heavily parasitized clams could be distinguished by observing gonads externally due to changing the gonad color from white to beige or brownish color. The color was due to the sporocysts' and rediae's brown color (Fig. 2).

Infection of gonads caused series of abnormalities to connective tissues, partial castration or complete castration (Figs. 5 and 6). Castration occurred in 15.1% of clams due to heavy infection and led to reproductive failure during spawning trials especially in May and June. Similar observations were recorded by Ramon et al. (1999) in *D. trunculus*. They recorded 8.4% prevalence (165 out of 1963) of sporocysts of *Baccigar baccigar* in *D. trunculus* in the Mediterranean coast of Spain. In that study, complete castration was observed in only 2.4% of the population which is less than that observed in the present study (15.1%). In the present study, 11%, 8% and 5% of the population were heavily infected in El-Max, Timsah Lake and Ezbet El-Borg, respectively. Castration was observed in almost 90% of the heavily infected specimens that had damaged follicles. Histological observations showed heterotrophy and disintegration of cells and lytic cell necrosis (Figs. 5D and 6D). Similarly, Infection with *Bucephalus* sp. caused reproductive failure and destroyed the ovaries of the pearl oysters, *Pinctada radiata* in the study of Khamdan (1998).

Infection prevalence and intensity with size

Percentage of infection observed from histological studies increased with clam size. Heavy infection that led to gonad castration was restricted to the bigger clams (shell length of ⩾31.9 mm) with ripe gonads. Clams with shell length ⩽20 mm showed the lowest presence of parasites. This may be attributed to less sexually mature individuals among these sizes since the study showed that infection was mainly localized in the gonads. Ramon et al. (1999) and El-Wazzan et al. (2012) showed that clam reach sexual maturity at shell length of 19 and 18.5 mm, respectively. Infection intensity in gonads ranged between light to severe infection. The light infection level by digenean larvae were restricted to female and male gonads, while severe infections were found to be spread beyond the gonads to infect other organs.

Similar results were observed in the study of Ramon et al. (1999), when prevalence was examined in different size classes, it was found that wedge clams with SL < 19 mm long were not affected whereas clams > 22 mm showed more infection prevalence. Clams at size 33–36 mm long, represented the highest prevalence (15–23% of the infected clams). Clams > 37 mm showed the least infection prevalence (Ramon et al., 1999).

Several theories have been suggested for the reason of increased infection with clam size. In addition to the sexual maturity hypothesis, the more abundance of final host during the period of high infection intensity was suggested by Nago and Choi (2004). Additionally, Ramon et al. (1999) related such relation to filtering larger amounts of water by bigger clams which increased the exposure to parasites.

In all cases, when spawning was attempted in clams during the period of high prevalence from April to June, spawning failed even during the spawning season when up to 100% of the clams examined in parallel study (El-Wazzan et al., 2012)

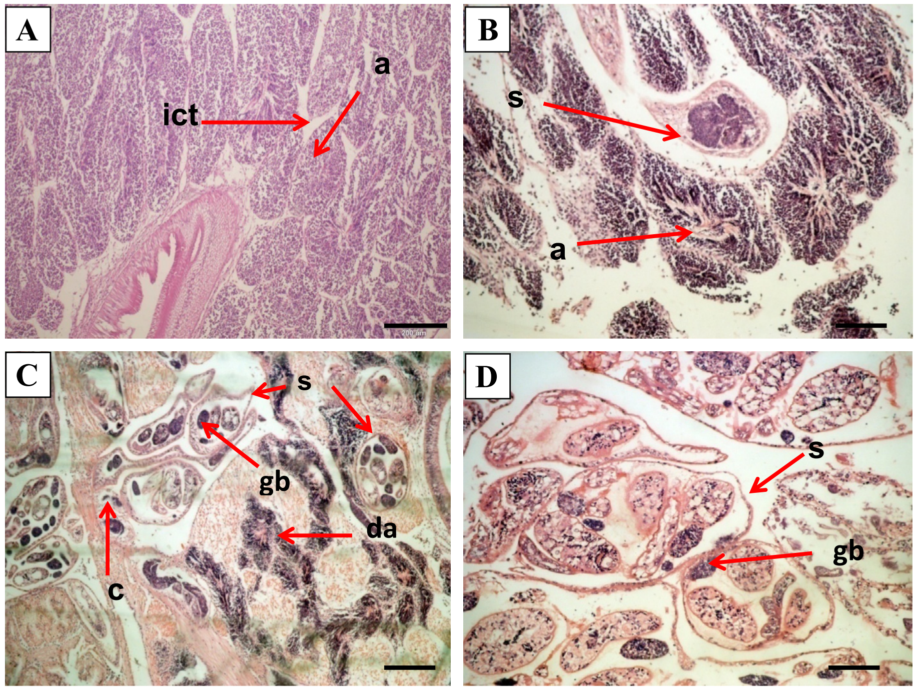

Figure 6 Photomicrographs of transverse sections through the testes of *Tapes decussatus*: (A) normal testis (scale bar = 200 μm), and testes infected with cercaria larvae: (B) Light infection (scale bar = 150 μm), (C) moderate infection (scale bar = 100 μm) and (D) heavy infection (scale bar = 50 μm). *Abbreviations:* a, acinus; ict, interacinar connective tissue; c, cercaria; da, degenerated acinus; gb, germinal ball; s, sporocyst.

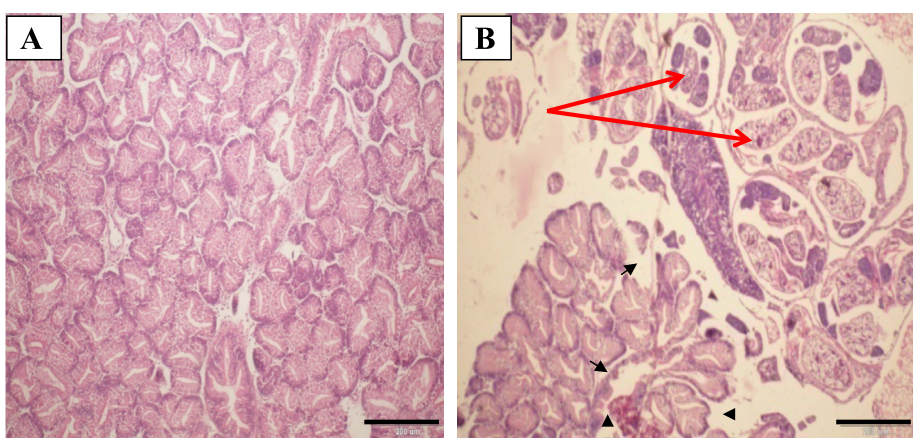

Figure 7 Photomicrographs of transverse section through the digestive gland of *Tapes decussatus* (A), normal uninfected gland; (B), shows gonad with heavy infections by cercaria larvae (arrows) that invaded the digestive gland causing autolysis (arrow heads). Scale bars = 200 μm.

were either ripe or spawning/partially spent which reflects the negative effect of these parasites on broodstock by decreasing fecundity or castration and complete reproductive failure. More than 90% of heavy parasitism targeted female gonads and destroyed ovaries.

Histopathological abnormalities in clam organ caused by parasites

Gonads. The normal ovary of *T. decussatus* collected from different stations consists of follicles with developing and mature oocytes (Fig. 5A). The interfollicular connective tissues are present around the follicles (Fig. 5A). In light and moderate infection, the larvae were found around the ovary and between the gonadal follicles (Fig. 5B and C). The interfollicular connective tissues, follicles and oocytes were degenerated in the heavy infection and resulted in ovary castration (Fig. 5D).

The normal testis of *T. decussatus* collected from different stations consists of acini with developing spermatocytes, spermatids, in addition to sperm in more developed testes. The acini are surrounded by interacinar connective tissue (Fig. 6A).

In light and moderate infection (Fig. 6B and C, respectively), the digenean larvae increased in number causing degeneration of connective tissues and acini shrinkage with reduced sperm number and degeneration of spermatocytes in some aci-

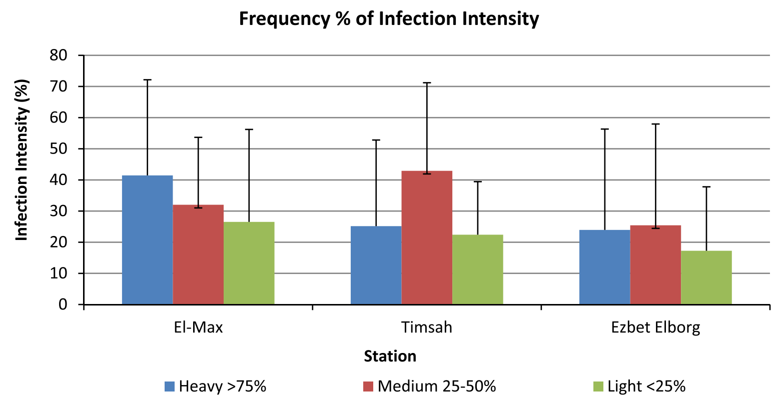

Figure 8 Average infection intensities (as% of infected clams) by trematode larvae of the carpet shell clam, *Tapes decussatus*, over a twelve months duration.

ni. In heavy infection (Fig. 6D), the acini, and connective tissue were replaced by the larvae and resulted in castration. Sex could not be differentiated in castrated clams (Fig. 6D).

The highest abnormalities in gonads over the study period were recorded in El Max (83.3%) while the lowest percentage of gonads abnormalities was recorded in Ezbet El-Borg, Damietta (33.3%) which showed the possible negative effect on reproduction and eventually total clam production.

Digestive gland. The digestive gland was also affected by parasites. The normal un-infected digestive gland of *T. decussatus*, consists of primary and secondary tubules with various degrees of vacuolization (Fig. 7A). In clams infected with parasites (Fig 7B) the digenean larvae replaced the gonads tissue and were found between the tubules and surrounded the digestive gland causing autolysis of digestive cells and increased vacuolization which led to reduction of function or complete loss of function with increased invasion by the parasite.

In conclusion, the data presented in the present study showed that the three study sites are considered highly contaminated with parasites. This might be one of the reasons of the declined clam population in these sites. This is considered as an important alarm for the Egyptian clam fisheries that requires continuous follow up and monitoring for the status of these fisheries to enhance future potential to eliminate such negative impacts for proper management of clam fisheries and grow-out sites in Egypt.

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